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13. ABSTRACT *(Maximum 200 words)*

The original sabbatical proposal was modified to two separate aims, both designed to acquire knowledge of genetics to be applied to mammary carcinoma. These studies were carried out at Stanford University in the laboratories of Stuart Kim, David Botstein and Pat Brown. First, in the Kim lab, a genetic screen was performed in C.elegans in a sensitized background (using worms mutant for Gap) to identify worms that missorted Let-23, the worm EGF receptor, in polarized vulva precursor cells. By complementation testing and STS mapping, a locus has been identified on chromosome 4 that results in missorting of Let-23 from the basolateral to apical surface. Second, microarray technology in the Botstein and Brown labs was utilized to identify sets of genes that are induced by antioxidants in mammary and colorectal carcinoma cells that culminate in p53-independent, p21-dependent apoptosis. Candidate genes have been identified and are being characterized. In addition, the latter experience enabled me to develop a microarray core lab at Vanderbilt University.

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Introduction: The goal of my sabbatical was to acquire a working knowledge of genetics. As has been previously discussed, logistical problems prevented the transfer of transgenic mice to Allan Balmain's laboratory at Onyx in Richmond, California This led to a major change in direction. Two projects were conducted that built on advances in my lab but were designed to harness the power of molecular genetics to propel the work in my lab ahead.

Body: The first project was performed in the laboratory of Stuart Kim in the Department of Developmental Biology at Stanford University. My lab has been studying the sorting and processing of mammalian EGF receptor ligands in mammalian polarized epithelial cells (1-3). In mammalian cells, EGF receptor is restricted to the basolateral surface. Ligand engagement of the EGF receptor initiates a signal transduction cascade that activates Ras, Raf and MAP kinase. We have found that TGFα (1) and amphiregulin (3) are delivered preferentially to the basolateral surface but then are processed differently. Multiple forms of amphiregulin are released and we are studying their possibly different biological roles. EGF (2) is delivered to both the basolateral and apical surface, but is preferentially cleaved by a metalloprotease-like enzyme in the basolateral compartment.

Dr. Kim's lab has focused on signaling events in polarized vulva precursor cells that result in a fully differentiated vulva (4). Lin-3, a TGFα homologue, binds to Let-23, an EGF receptor homologue, in the basolateral compartment of polarized vulva precursor cells in the second larval stage of the worm (5, 6). This initiates a signal transduction cascade that activates Ras and MAP kinase which results in a fully differentiated vulva. The Kim lab has identified three mutants that result in missorting of this worm EGF receptor from the basolateral to the apical compartment and the worms are no longer able to form a vulva (7-9). Mutations of three PDZ-containing proteins (Lin-2, Lin-7 and Lin-10) are responsible for this phenotype. Mammalian homologues of these proteins have been identified, and, in at least with Lin-2, it appears to be a tumor suppressor gene.

I carried out a mutant screen in worms that were mutant in Gap, a gene important in inactivation of active Ras (10). These worms had no observable phenotype. In this sensitized background, I identified 10 mutants that resulted in a multivulva. By immunohistochemistry, the worm EGF receptor appeared to be misdirected to the apical compartment. Complementation tests revealed that this mutant was not due to any of the previously characterized PDZ-containing proteins. STS mapping was carried out and I found that the locus mapped to chromosome 4 (11). More refined mapping has narrowed the region on chromosome 4 to 1.5 map units between stP51 and stP35 . I intend to carry out Yac injections and deficiency mapping to identify the gene responsible for this phenotype. The mammalian homologue then will be identified and its role in mammary carcinoma will be studied.

The second project was a follow-up of an important clinical observation that we have made recently. That is, that antioxidants enhance the anti-tumor efficacy of cytotoxic chemotherapy in mammary and colorectal cancer cells *in vitro* and *in vivo* (12). Furthermore, we have elucidated a molecular mechanism by which one of these antioxidants, PDTC, acts (13). This involves activation of protein kinase A which

phosphorylates serine^{299} in C/EBPβ that translocates to the nucleus, binds to a p53-independent site in the p21 promoter to induce apoptosis. I was exposed to microarray technology in the labs of David Botstein and Pat Brown who pioneered this technology (14-16) at Stanford University and employed it to identify genes that are expressed following administration of PDTC. We have examined the effect of PDTC on the expression of 5,000 genes in mammary and colorectal cancer cells. These studies should allow us to identify possible additional targets of the action of PDTC. We are now setting up a microarrayer at Vanderbilt University and intend to utilize it to identify molecular events in rodent and human mammary carcinoma It is anticipated that microarray technology, coupled with laser capture microdissection, will be utilized to identify genetic events in this model and the results will provide insights into the molecular pathogenesis of human mammary carcinoma.

Conclusions: In summary, I accomplished my goal for the sabbatical, which was to acquire a working knowledge of genetics. Stanford University provided an ideal environment for this work. This knowledge will be applied to elucidating molecular events underlying the pathogenesis of mammary carcinoma, as well as to formulate new therapeutic approaches to mammary and colorectal cancer. . For example, we have observed that there is accelerated mammary tumor formation in mice bigenic for TGFα and c-neu (17) and, more recently, that an EGF receptor tyrosine kinase inhibitor blocks tumor formation in these bigenic mice (18). We have developed a novel treatment for mammary and colon cancer by blocking the EGFR and inhibiting the enzyme that cleaves cell surface TGFα to release the mature soluble growth factor (19). A recent manuscript from our group demonstrates that inhibition of the Ras pathway with farnesyltransferase inhibitors may be useful in the treatment of mammary cancer due to sustained overexpression of TGFα (20). Finally, the lessons that I learned during my sabbatical enabled me to compete successfully for the Mouse Models of Human Cancer Consortium; a major focus of the proposal is to develop mouse models of mammary cancer by downregulating or eliminating the type II TGFβ receptor in the mouse mammary gland.

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